31. The method as claimed in Claim 30, wherein the expression vector has a promoter and terminator that specifically function in *Bifidobacterium longum*.

32. The method as claimed in Claim 31, wherein the promoter is a nucleotide sequence consisting of 1- to 192-positions in SEQ ID NO: 1 and the terminator is a nucleotide sequence consisting of 472 to 600 of SEQ ID NO: 1.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Applicants have submitted a verified English translation of the certified Priority Document to perfect Applicants' claim for foreign priority. In light of such a submission, the present application is now entitled to the priority date of September 21, 2000.

The specification has been carefully reviewed and editorial changes have been effected. All of the changes are minor in nature and therefore do not require extensive discussion. Specifically, the specification headings have been amended in conformance with U.S. practice.

Claims 1, 2 and 26 have been cancelled without prejudice, claims 3, 4, 12-22, 24 and 25 have been amended, and new claims 28-32 have been added. The claim amendments and new claims have been presented to put the claims in better and proper form under U.S. practice, to more particularly define the present invention, and to further protect other specific embodiments

of the present invention. Support for the claim amendments and new claims is readily apparent from the teachings of the specification and the original claims.

With regard to the objection to the Abstract, Applicants have amended the term "said"on page 71, line 9, of the specification, to the term "the" to alleviate the Examiner's concerns.

With regard to the objection to the disclosure, Applicants wish to clarify that the spaces between the words "spectomycin", "resistance" and "The" are just spaces caused by full justification of the left and right margins. Thus, no words are missing from the specification.

With regard to the objections of claims 12-22 and 24-26, these objections are believe to be overcome by the amendments to the claims. Specifically, claims 12-21, 24 and 25 have been amended to put the claims in proper multiple dependent form. Further, claim 22 has been amended to direct to "*A bacterium Bifidobacterium longum 105-A/pBLES100-S-eCD (FERM BP-7274)* which Applicants believe addresses the Examiner's concerns. Lastly, claim 26 has been cancelled without prejudice.

With regard to the objection of claim 4, Applicants request the Examiner to hold this objection in abeyance until after an indication of allowable subject matter since it is possible that non-elected species might be rejoined later in prosecution.

With regard to the drawings, Applicants wish to note that revised formal drawings were filed on May 29, 2002. Applicants request that the Examiner review the application file and confirm receipt of the drawings.

With regard to the rejection of claims 1-5, 8-11 and 26 under 35 USC §112, first paragraph, this rejection is deemed to be untenable in view of the amendments to the claims and is

thus respectfully traversed. The claims have been amended to direct to the specific *Bifidobacterium* species of *Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium pseudolongum, Bifidobacterium thermophirum, Bifidobacterium breve, and Bifidobacterium infantis*. Clearly, any of these bacteria are commercially available or easily obtainable from the depository organizations. One of ordinary skill in the art is able to practice the claimed invention by using and operating these bacteria with fundamental procedures and methodologies of genetic engineering and/or biological engineering well known in the art (see commercial books on experiments described in the specification). It is well understood under U.S. practice that claims are not read in a vacuum but in light of the teachings of the specification and the knowledge in the art.

Thus, in view of the amendments to the claims and the comments above, this rejection can no longer be sustained and should be withdrawn.

With regard to the rejection of claim 22 under 35 USC §112, first paragraph, this rejection has been overcome by the filing of a Deposit Declaration enclosed herewith.

With regard to the rejection of claims 1-3 under 35 USC §112, second paragraph, this rejection has been overcome by the cancellation of claims 1 and 2, and the amendments to claim 3. Specifically, claim 3 has been amended to clarify the term "system" as comprising a bacterium transformed with a recombinant DNA for delivering said DNA specifically to tumor tissues under anaerobic conditions.

Thus, in view of the changes to the claims, this rejection can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1-3, 8-9 and 26 under 35 USC §102(a) as being anticipated by Yazawa et al. (IDS, Cancer Gene Therapy, Vol. 7, pp. 269-274), this rejection has been overcome by the cancellation of claim 26 and the filing of a Rule 1.131 Declaration enclosed herewith. As the Examiner can see from the enclosed Declaration, the Declaration clearly establishes conception, due diligence and reduction to practice of the claimed invention prior to the March 27, 2000 publication date of Yazawa et al. Although the document used to establish the date of the invention is the same as the cited Yazawa et al. reference, the listed authors of the document comprise five out of the seven inventors for the present application. Thus, the experiments and laboratory work performed by these inventors as represented in the supporting document clearly establish conception, due diligence and reduction to practice prior to the publication date of March 27, 2000 since the document was submitted for publication on January 14, 1999 and accepted on May 31, 1999. Consequently, since in light of the Declaration, Yazawa et al. is no longer a valid prior art reference under 35 USC §102(a), this rejection should be withdrawn.

With regard to the rejection of claims 1-4, 8, 9 and 26 under 35 USC §102(a) as being anticipated by Babincova et al. (Life and Medical Sciences Online, http://www.itrust.de/lamso/lpext.dll.Infobase0?title0003.htm?fn=docu 8/7/2000, pp. 1-4), this rejection has also been overcome by the same filed Rule 1.131 Declaration. Since Babincova et al. has a publication date of August 7, 2000 which is after the date of January 14, 1999 establish by the Declaration, Babincova et al. is also no longer a valid prior art reference under 35 USC §102(a). Thus, this rejection also cannot be maintained and should be withdrawn.

With regard to the rejection of claim 26 under 35 USC §102(b) as being anticipated by Matsumura et al. (IDS, Biosci. Biotech. Biochem., Vol. 61, pp. 1211-1212, 1997), this rejection has been render moot by the cancellation of the rejected claim.

With regard to the rejection of claims 1-5, 8, 9 and 26 under 35 USC § 103(a) as being unpatentable over Babincova et al. (Life and Medical Sciences Online, http://www.itrust.de/lamso/lpext.dll.Infobase0?title0003.htm?fn=docu 8/7/2000, pp. 1-4) in view of Tagliabue et al. (WO 96/11277), this rejection has also been overcome for the same reasons as noted above.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows.

3. (Amended) A method for delivering a gene in a system for delivering a DNA specifically to tumor tissues under anaerobic conditions, said system comprising a bacterium selected from the group consisting of *Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium pseudolongum, Bifidobacterium thermophirum, Bifidobacterium breve, Bifidobacterium infantis,* wherein ~~a~~said bacterium belonging to the genus *Bifidobacterium* and transformed with a recombinant DNA having said DNA is used as a gene delivery vector and the DNA delivered specifically to tumor tissues under anaerobic conditions is expressed in the tumor tissues.

4. (Amended) The method as claimed in ~~any one of Claims 1 to~~Claim 3, wherein the DNA is selected from the group consisting of:

(a) DNA coding for a protein having an antitumor activity, and

(b) DNA coding for a protein having an activity of converting a precursor of an antitumor substance into the antitumor substance.

12. (Amended) The method as claimed in any one of Claims ~~1~~3 to 11, wherein the bacterium is *Bifidobacterium longum*.

13. (Amended) The method as claimed in any one of Claims ~~1 to~~3, 4 or 6 to 12~~1~~, wherein the bacterium is *Bifidobacterium longum* -105-A/pBLES100-S-eCD (FERM BP-7274).

14. (Amended) A method for expressing a gene coding for a protein having an antitumor activity in tissue tumors specifically, which comprises use of the bacterium as claimed in any one of Claims ~~1~~3 to 5 or 8 to 12~~1~~1.

15. (Amended) A method for expressing a gene coding for a protein having the activity of converting a precursor of an antitumor substance into the antitumor substance in tissue tumors specifically, which comprises use of the bacterium as claimed in any one of Claims ~~1 to~~3, 4 or 6 to 12~~1~~1.

16. (Amended) A pharmaceutical composition comprising the bacterium as claimed in any one of Claims ~~1~~3 to 13~~1~~1.

17. (Amended) ~~The pharmaceutical composition as claimed in Claim 16, wherein the~~A pharmaceutical composition ~~comprises~~comprising a combination of the bacterium as claimed in any one of Claims ~~1 to~~3, 4 or 6 to 13~~1~~1 and the precursor of an antitumor substance.

18. (Amended) ~~The pharmaceutical composition as claimed in Claim 16, wherein the~~A pharmaceutical composition ~~comprises~~comprising the bacterium as claimed in any one of Claims ~~1 to~~3, 4 or 6 to 13~~1~~1 and the precursor of an antitumor substance.

19. (Amended) The pharmaceutical composition as claimed in ~~any one of~~ Claims 16 ~~to 18~~, wherein the bacterium is *Bifidobacterium longum*.

20. (Amended) The pharmaceutical composition as claimed in ~~any one of Claims 16 to~~ Claim 19, wherein bacterium is *Bifidobacterium longum* 105-A/pBLES100-S-eCD (FERM BP-7274).

21. (Amended) A bacterium belonging to the genus *Bifidobacterium*, which is used in the method as claimed in any one of Claims ~~1~~3 to 13~~1~~.

22. (Amended) A bacterium *Bifidobacterium longum* 105-A/pBLES100-S-eCD (FERM BP-7274).

24. (Amended) A method of treating a solid tumor, which comprises use of the method as claimed in any one of Claims ~~1~~3 to 15~~1~~.

25. (Amended) A method of treating a solid tumor, which comprises administering the bacterium as claimed in any one of Claims ~~1 to~~3, 4 or 6 to 13~~1~~ in combination with the precursor of an antitumor substance.

Version with Markings to
Show Changes Made

~~SPECIFICATION~~

ANAEROBIC BACTERIUM AS A DRUG FOR CANCER GENE THERAPY

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to anaerobic bacteria belonging to the genus *Bifidobacterium* useful for gene therapy of solid tumors, a pharmaceutical composition containing the same, a method of delivering a gene and a method of treating solid tumors by use of the same.

2. Description of the ~~Prior~~ Related Art

Hypoxic regions are characteristic of solid tumors in animal (Int. J. Radiat. Oncol. Biol. Phys., 10: 695-712 (1984)) and occur with high frequency in many types of human solid tumors (Fischer-Verlag, stuttgart, 219-232 (1994), New York). Tissue oxygen electrode measurements (i.e. a membrane examination device capable of measuring dissolved oxygen) taken in cancer patients have shown a median range of oxygen partial pressure of 10 to 30 mmHg in tumors, with a significant proportion of readings b low 2.5 mmHg, whereas those in normal tissu s range from 24 to 66 mmHg.

Accordingly, gene therapy in solid tumors that targets

from *B. longum*. Amp^r represents an ampicillin resistance gene, Ter^r represents a tetracycline resistance gene and Ori represents an origin of replication.

Fig. 11 shows a process for constructing plasmid vector pBLES100-S-eCD in which the CD gene derived from *Escherichia coli* was integrated, which is used as expression vector for *B. longum*.

~~DETAILED~~ DESCRIPTION OF THE ~~INVENTION~~ PREFERRED EMBODIMENTS

The present invention provides bacteria belonging to the genus *Bifidobacterium* (abbreviated hereinafter to the bacteria of the genus *Bifidobacterium*) having a gene coding for a substance having an antitumor activity. Preferably said substance has a higher antitumor activity than in its parent strain.

The substance having a higher antitumor activity than in its parent strain is e.g. the substance which is expressed in a larger amount than in its parent strain, has an improvement in Km value as compared with the counterpart (enzyme) expressed in its parent strain, or is hardly degradable than in the counterpart expressed in its parent strain, resulting in the higher activity. The parent strain in the microbiology usually m ans the wild stain, from which substains, clon s and mutants and the like are derived (Biologic dictionary, third edition, tokyokagakudoujin 1998).

105-A/pBLES100-S-eCD, th tumor-bearing mice were sacrificed, and the concentration of 5-FU in the tumor tissues in the thighs was examined. For measurement of the concentration of 5-FU, the tumor tissues to which the transformant *B. longum* 105-A/pBLES100-S-eCD had been topically injected, and the tumor tissues to which the transformant had not been injected were excised, and the concentration of 5-FU in the tumor tissues was measured by GC-MS method (J. Chromatography, 564, 137 (1991)) in Otsuka Assay Laboratories.

As a result, only about 10.0 ng/g 5-FU could be detected in the tumor tissues to which *B. longum* 105-A/pBLES100-S-eCD had not injected, while 588.8 ng/g 5-FU was detected in the tumor tissues to which *B. longum* 105-A/pBLES100-S-eCD had been topically injected.

From the results described above, it was confirmed that systemically administered 5-FC is converted into 5-FU in tumor tissues specifically.

~~INDUSTRIAL APPLICABILITY~~

The present invention provides a method of expressing a substance having an antitumor activity or a converting enzym in tumor tissues specifically under anaerobic conditions by using, as gene delivery vectors, anaerobic bacteria belonging to the genus *Bifidobact rium*, some of which are domestic in human intestine and nonpathogenic bact ria, as w ll as transformed or mutated bacteria belonging to the genus

ABSTRACT OF THE DISCLOSURE

The present invention provides a bacterium belonging to the genus *Bifidobacterium*, by which DNA coding for a protein having an antitumor activity or DNA coding for a protein having the activity of converting a precursor of an antitumor substance into the antitumor substance is delivered to tumor tissues specifically under anaerobic conditions thereby expressing the protein encoded by the DNA, as well as a pharmaceutical composition comprising ~~said~~ the anaerobic bacterium.